

SULFIDE REMOVAL FROM PETROLEUM  
REFINERY WASTEWATER USING  
*Pseudomonas putida* (ATCC 49128) AND *Bacillus*  
*cereus* (ATCC 14579)

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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SULFIDE REMOVAL FROM PETROLEUM REFINERY WASTEWATER USING  
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MANI MALAM AHMAD

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## ABSTRAK

Hidrogen sulfida ( $\text{H}_2\text{S}$ ) dikenali sebagai salah satu daripada bahan pencemar berbahaya dari air sisa penapisan petroleum (petroleum refinery based wastewater, PRW), yang dilaporkan dalam kes persekitaran dan ekonomi, di samping komplikasi kesihatan terhadap manusia. Sifat semulajadi heterogen PRW berserta kekurangan pendekatan klasik mitigasi fizikokimia menjadi sebab pencarian alternatif yang efektif kos dan endah alam sekitar dengan kesan negatif yang minimum. Kultur bakteria bercampur (bacterial mixed culture, BMC) terintegrasi baru, *Pseudomonas putida* (ATCC 49128) dan *Bacillus cereus* (ATCC 14579) dengan kebolehan biodegradasi terhadap PRW kompleks dan berkepekatan tinggi dicadangkan sebagai alternatif pengoksidaan sulfida yang mampu menangani kekurangan tersebut. Kesan saling melengkapi tersebut mungkin menjadi punca toleransi terhadap kesan rencatan  $\text{H}_2\text{S}$ . Untuk permulaan, kesan kepekatan nutrient dan keadaan fizikal operasi lain iaitu suhu, pengadukan, dan masa aklimatisasi terhadap pertumbuhan penciran tersebut disaring dan seterusnya dioptimum di dalam kelalang goncang dengan bantuan perisian Design Expert. Berdasarkan keadaan pertumbuhan optimum, bandingan RE BMC berserta kultur tulen dijalankan menggunakan air sisa disimulasikan pada kepekatan berbeza (200, 300 dan 500 ppm). Nilai RE model kultur tulen dan bercampur didapati pada 95%–100% dengan menggunakan air sisa disimulasikan dalam kelalang goncang. Walau bagaimanapun, nilai RE mantap ditunjukkan oleh model terpilih (BMC) dengan nilai RE tertinggi pada 99% bagi 500 ppm  $\text{S}^{-2}$  dalam tempoh 24 jam HRT. Tambahan lagi, kesan bersinergi parameter proses kultur kelompok terhadap pengoksidaan sulfida menggunakan BMC dioptimumkan melalui kaedah statistik menggunakan kaedah reka bentuk komposit pusat berpusatkan muka tiga aras (face-centered central composite design, FCCCD) dan kaedah permukaan gerak balas (response surface methodology, RSM). Nilai RE tertinggi pada 91% dicapai melalui model BMC pada parameter proses yang optimum 500 ppm, 0.5 vvm, 30 °C, dan 140 rpm masing-masing bagi kepekatan sulfida influen, pengudaraan, suhu, dan pengadukan, dalam tempoh 8 jam HRT. Tambahan pula, keupayaan biodegradasi sulfida oleh BMC disahkan menggunakan PRW sebenar berdasarkan parameter optimum dengan nilai RE yang tinggi pada 98.76%–99.75%. Sebagai kesimpulan, dapatan kajian ini dapat mengisi kelompongan berkaitan pengoksidaan sulfida dalam PRW rekalsitrasi secara biologi oleh model BMC baru dalam bioreaktor kultur aerobik kelompok skala makmal. Untuk itu, keandalan dan kebolehan peramalan model endah alam sekitar ini adalah sesuai untuk merawat air sisa sarat sulfida dengan potensi keupayaan RE yang maksimum.

## ABSTRACT

Hydrogen sulfide ( $\text{H}_2\text{S}$ ) has been recognized as one of the hazardous pollutants from petroleum refinery based wastewater (PRW), with reported cases of environmental and economic challenges, besides other human health complications. The heterogeneous nature of PRW couple with the documented shortfalls of the classical physicochemical mitigation approaches was behind the quest for a cost-effective and eco-friendly alternative with minimum adverse effects. A novel integrated bacterial mixed culture (BMC), *Pseudomonas putida* (ATCC 49128) and *Bacillus cereus* (ATCC 14579) with traceable imprints in biodegradation of high-strength PRW proposed as a suitable alternative sulfide oxidation approach with a potential solution to the shortfalls. Their complementary helper effects could be the reason behind their tolerance to the  $\text{H}_2\text{S}$  inhibitory effect. Initially, the impact of nutrient concentration and other physical operating conditions of temperature, agitation and acclimatization time to the isolates growth were screened and subsequently optimized in shake flasks with the aid of Design-Expert software. Based on these optimized growth conditions, relative removal efficiency RE of BMC along with the corresponding pure cultures were carried out using simulated wastewater at different concentrations (200, 300 and 500 ppm). The RE of the pure and mixed-culture models was found to be 95%–100% with simulated wastewater in shake flasks. However, a sustained RE was displayed by the selected model (BMC), with the highest RE of 99% in 500 ppm of  $\text{S}^{-2}$  within the HRT of 24 hours. Furthermore, the synergistic contribution effects of the batch culture process parameters on the BMC sulfide oxidation were optimized at three coded levels using face-centered central composite design (FCCCD) and response surface methodology (RSM) statistical tools. The highest RE of 91% was obtained with the BMC model at optimum process parameters of influent sulfide concentration, aeration, temperature, and agitation of 500 ppm, 0.5 vvm, 30 °C, and 140 rpm, respectively, within 8 hours of HRT. Moreover, the sulfide biodegradation capability of BMC was validated using the actual PRW based on the optimum parameters which resulted in overwhelming RE values of 98.76%–99.75%. Conclusively, the findings fill in some gaps concerning the biological sulfide oxidation from recalcitrant PRW by a novel BMC model in a lab-scale batch culture aerobic bioreactor. It suggested that the reliability and prediction ability of this eco-friendly model is therefore suitable for treating high-strength sulfide-laden wastewater with potential capabilities of maximum RE.

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## LISTS OF SYMBOLS

L/min	Liter per minute
vvm	Vessel volume per minute
°C	Degree Centigrade
rpm	Revolution par minute
hr	hour
ppm	Part per million
mg/L	Milligram per liter
wv <sup>-1</sup>	Weight-volume
vv <sup>-1</sup>	volume-by-volume
keV	Accelerating voltage
X <sub>t</sub>	Cell biomass at time t
X <sub>0</sub>	Initial cell biomass
ln	Natural logarithm
log	Logarithm
S <sub>t</sub>	Sulfide concentration at time t
S <sub>0</sub>	Initial sulfide concentration
ΔS	Change in sulfide concentration
e	exponential
μ	Specific growth rate
t	time
β	Beta

## LIST OF ABBREVIATIONS

ACWW	Actual Waste Waster
AGWW	Augmented Waste Water
AC	Activated Carbon
AD	Anaerobic Digester
AF	Accuracy Factor
ANOVA	Analysis of Variance
APHA	American Public Health Association
ASFBR	Anaerobic Structured Fluid-Bed Reactor
ATCC	American Type Culture Collection
ATSDR	Agency for Toxic Substances and Disease Registry
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
APAT	APS: Phosphate adenylyltransferase
APS	Adenosine 5' phosphosulfate
ATP	Adenosine triphosphate
BAS	Biofilm airlift suspended reactor
BF	Bias Factor
BIS	Bureau of Indian Standard
BMC	Bacteria Mixed Culture
BOD	Biological Oxygen Demand
BRT	Batch reactor Type
BTEX	Benzene, Toluene, Ethylbenzene and Xylene
BSO	Biological Sulfide Oxidation
COD	Chemical Oxygen Demand
C <sub>O2</sub>	Oxygen Concentration
CSTR	Continuously Stirred-Tank Reactor
CDW	Cell Dry Weight
CV	Coefficient of Variability
DSU	Desulfurization Unit
DF	Dilution Factor
DMDS	Dimethyl disulfide

DoE	Design of Expert
DSR	Denitrifying Sulfide Removal
DO	Dissolved Oxygen
DOE	Design of Experiments
EC	Electrical Conductivity
EDXS	Energy Dispersive X-ray Spectroscopy
EBRT	Empty Bed Residence Time
FFD	Fractional Factorial Design
FCCCD	Face-Centered Central Composite Design
HSPs	Heat Shock Proteins
HRT	Hydraulic Retention Time
HS <sup>-</sup>	Hydrogen Sulfide Ion (Bisulfide)
H <sub>2</sub> S	Hydrogen sulfide
IDLH	Immediately Dangerous to Life or Health
ICMR	Indian Council of Medical Research
LOAEL	Lowest-Observed-Adverse-Effect Level
MABR	Membrane Aerated Biofilm Reactor
MDU	Microaerobic Desulfurization Unit
NISDWR	National Interim Secondary Drinking Water Regulation
N	Nitrogen
NA	Nutrient Agar
NB	Nutrient Broth
NIOSH	National Institute for Occupational Safety and Health
NO <sub>3</sub> <sup>-</sup>	Nitrate
OFAT	One-factor-at-a-time
OVAT	One-variable-at-a-time
OLR	Organic Loading Rate
OTR	Oxygen Transfer Rate
OUR	Oxygen Utilization rate
ORP	Oxidation-Reduction Potential
O <sub>2</sub>	Oxygen
OEL	Occupational Exposure Limit
OD	Optical Density

OSHA	Occupational Health and Safety Agency
PRW	Petroleum Refinery Wastewater
PEL	Permissible Exposure Limit
PAH	Polycyclic Aromatic Hydrocarbon
pH	Potential of Hydrogen
PDO	Persulfide dioxygenase
PPD	Personal Protection Device
PPE	Personal Protection Equipment
PTCC	Persian Type Culture Collection
PRESS	Predicted Residual Error Sum Square
R1/R2	Reaction Rate
RE	Removal Efficiency
RFD	Reference Dose
RFC	Reference Concentration
RpoS/H	RNA Polymerase sigma gene S/H
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
S <sup>2-</sup>	Sulfide ion
SOB	Sulfide Oxidizing Bacteria
SRB	Sulfate Reduction Bacteria
S <sub>2</sub> O <sub>3</sub> <sup>-2</sup>	Thiosulfate
S <sup>0</sup>	Elemental sulfur
SO <sub>3</sub> <sup>2-</sup>	Sulfite
SO <sub>4</sub> <sup>2-</sup>	Sulfate
SQR	Sulfite Quinine Reductase
SOX	Sulfide Oxidizing gene
SCR	Sulfide Cytochrome Reductase
SLR	Sludge Loading rate
STEL	Short-time Exposure Limit
TOMES	Thiosulfate-Oxidizing Multi-Enzyme System
TVL	Threshold Value Limit
TDS	Total Dissolved Solid
TSS	Total Suspended Solid

VFAs	Volatile Fatty Acids
VSLR	Volumetric Sulfide Loading Rate
VSS	Volatile Suspended Solid
$V_{up}$	Upflow Velocity
UASB	Upflow Anaerobic Sludge Bed
WHO	World Health Organization
WQI	Water Quality Index
WWTPs	Waste Water Treatment Plants

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